



**KnE Life Sciences**

  
**Knowledge E**  
Engaging minds

## The Veterinary Medicine International Conference (VMIC)

12-14 July 2017

ISSN 2413-0877



[www.KnEpublishing.com](http://www.KnEpublishing.com)

## The Veterinary Medicine International Conference (VMIC)

VMIC—The Veterinary Medicine International Conference—is one of the world's leading conference focusing on a wide array of topics including Veterinary Medicine and Biomedical Science. It offers a stimulating venue for scientists, researchers, lecturers, general practitioners, and others to broaden their social scientific network. This conference contributes to improving human and public health by improving agricultural and food systems, advancing biomedical and comparative medical research, preventing and addressing zoonotic diseases, kit diagnostic, enhancing environmental and ecosystem health, and helping manage the 21st-century public health challenges.

**Conference date:** 12–14 July 2017

**Location:** Surabaya, East Java, Indonesia

**Editors:** Sri Agus Sudjarwo, Fedik A. Rantam, rer. nat. Gunawan Indrayanto, Muchammad Yunus, Rimayant, Wiwik Misaco, Tita Damayanti Lestari, Mustofa Helmi Effendi, Dikky Eka Mandala Putranto, and Shafia Khairani

**Organizer:** Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia

**Sponsors:** Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Universitas Airlangga, ROMINDO PRIMAVETCOM, Gadjah Mada University, Bogor Agriculture Institute, ILRI (International Livestock Research Institute), Kagoshima University, Miyazaki University, Faculty of Veterinary Science, Chulalongkorn University, College of Veterinary Medicine, Tarlac Agricultural University, Yamaguchi University, Erasmus MC, Kasetsart University, Wyndham Veterinary Clinic, GERBU – Germany, USAID

**Published:** 29 November 2017

**ISSN:** 2413-0877

## Table of Contents

[The Veterinary Medicine International Conference \(VMIC\) | pages 1-9](#)

[\*\*Toll-Like Receptors \(TLRs\) Play Role in Adaptive Immunity in Rabbits Immunized by \*Sarcoptes scabiei\* Proteins\*\*](#)

Nunuk Dyah Retno Lastuti, Fedik Abdul Rantam, Poedji Hastutiek, Dony Chrismanto  
[The Veterinary Medicine International Conference \(VMIC\) | pages 10-20](#)

[\*\*Preservation Effect of Grouper \(\*Epinephelus\* sp\) Fillet Against Survival of Anisakidae\*\*](#)

Hartanto M. Raharjo, Setiawan Koesdarto, A.T Soelih Estoepangestie, Kusuma Wardhani L.D  
[The Veterinary Medicine International Conference \(VMIC\) | pages 21-27](#)

[\*\*Acanthocephalan in \*Xenochrophis piscator\* Snake in Sidoarjo Indonesia\*\*](#)

Inggarsetya Syah Audini, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto  
[The Veterinary Medicine International Conference \(VMIC\) | pages 28-33](#)

[\*\*The Identification Blood Parasites On Pig \(\*Susdomesticus\*\) In Polewali Mandar District\*\*](#)

Silvana Arfin, Lucia Muslimin, Adryani ris  
[The Veterinary Medicine International Conference \(VMIC\) | pages 34-40](#)

[\*\*Spirometra in \*Ptyas mucosus\* Snake in Sidoarjo, Indonesia\*\*](#)

Garindra Tiara Pranashinta, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto  
[The Veterinary Medicine International Conference \(VMIC\) | pages 41-47](#)

[\*\*Toxicity of \*Citrus mitis\*, \*Citrus aurantifolia\*, and \*Citrus maxima\* leaf extract toward mortality of \*Aedes aegypti\* larvae \(Diptera: Culicidae\)\*\*](#)

Hamidah Hamidah, Hebert Adrianto  
[The Veterinary Medicine International Conference \(VMIC\) | pages 48-61](#)

[\*\*An In Vitro Antibacterial Activity Test of Meniran Herbs' \(\*Phyllanthus Niruri\* L.\) Ethanol Extract Against \*Mycoplasma gallisepticum\* causes Chronic Respiratory Disease \(CRD\) in Broiler Chickens\*\*](#)

Emy Koestanti Sabdoningrum, Sri Hidanah, Retno Sri Wahjuni, Sri Chusniati, Arimbi Arimbi  
[The Veterinary Medicine International Conference \(VMIC\) | pages 62-68](#)

[\*\*Bioremediation of Mercury \(II\) Contaminated Seawater Using the Diatom \*Skeletonema costatum\*\*\*](#)

Thin Soedarti, Tini S., Sucipto H., Eko P. Kuncoro  
[The Veterinary Medicine International Conference \(VMIC\) | pages 69-76](#)

[\*\*BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application\*\*](#)

Theresia Indah B., Bambang Sumaryono, Ketut Suardita, Amelia Putri R.  
[The Veterinary Medicine International Conference \(VMIC\) | pages 77-83](#)

[\*\*Inhibition of Apoptosis in Retinal of Newborn Mice Due to Congenital Toxoplasmosis\*\*](#)

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti



[The Veterinary Medicine International Conference \(VMIC\) | pages 84-92](#)

**Effect of Spirulina Platensis on The Number of Spermatogenic Cells in The Seminiferous Tubules of Rat (Rattus Norvegicus) with Excessive Physical Exercise**

Rahmah Wahyu Rosidawati, Rimayanti Rimayanti, Koesnoto Supranianondo  
[The Veterinary Medicine International Conference \(VMIC\) | pages 93-104](#)

**Phytochemicals, Antioxidant and Antifungal Properties of Acorus calamus, Curcuma mangga, and Allium sativum**

Bayyinatul Muchtaromah, Mujahidin Ahmad, Emy Koestanti S, Yuni Ma'rifatul A, Velayati Labone A  
[The Veterinary Medicine International Conference \(VMIC\) | pages 105-110](#)

**Shark Species on Export Products from East Java and Bali by Dna Barcoding Based on Internal Transcribed Spacer-2 (Its-2) Locus in Mitochondrial**

Eduardus Bimo Aksono  
[The Veterinary Medicine International Conference \(VMIC\) | pages 111-124](#)

**Bioactivity of human Menopausal Gonadotrophin (hMG) and Deglycosylated hMG (hMGdG) from Urine of Post-Menopausal Women On invitro Bovine Embryonic cleavage**

Herry Agoes Hermadiv  
[The Veterinary Medicine International Conference \(VMIC\) | pages 125-138](#)

**The Effect of Frequency Acoustic Stimulation Sound on Intrauterine Weakening of Pregnant Sheep**

Djamil Suherman, Hermanto Tri Joewono, I Komang Wiarsa Sardjana  
[The Veterinary Medicine International Conference \(VMIC\) | pages 139-152](#)

**The Potency of  $\Delta F$  508-T Gen Mutant the Coding of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) As Prototype at the Congenital Bilateral Absence of Vas Deferens (CBAVD) Disease in Indonesia**

Maslichah Mafruchati, Mas'ud Harijadi, Widjiati Widjiati, Boerhan Hidayat  
[The Veterinary Medicine International Conference \(VMIC\) | pages 153-165](#)

**A Retrospective Study of Canine Pyometra in Segar Veterinary Hospital, Kuala Lumpur, Malaysia Year 2012-2016**

Ng Xin Hui, Mas'ud Hariadi, Hardany Primarizky  
[The Veterinary Medicine International Conference \(VMIC\) | pages 166-174](#)

**Oocyte Quality and Subsequent In Vitro Maturation of Sheep Oocyte-Cumulus Complex from Ovary with Presence and Absence of Corpus Luteum**

Rini Widyastuti, Mas Rizky A.A. Syamsunarno, Takdir Saili, Arief Boediono  
[The Veterinary Medicine International Conference \(VMIC\) | pages 175-188](#)

**The Effect of Insulin-Like Growth Factor-I of Pregnant Crossbred Mare Serum and Insulin-Like Growth Factor-I Recombinant Mouse on Estrous Cycles and Litter Sizes of Mice (Mus musculus)**

Tjuk Imam Restiadi, Imam Mustofa, Suzanita Utama, Sri Mulyati  
[The Veterinary Medicine International Conference \(VMIC\) | pages 189-196](#)

**Adding of L-Arginin Amino Acidin Skim Milk Diluent to Maintain Quality of Buck Sperm in Cold Temperature**

Tri Wahyu Suprayogy, Suhermi Susilowati, Tatik Hernawati  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 197-204

**Profile of Crude Protein Tyrosine Kinase on Plasma Membrane of Merino Sheep Spermatozoa Using the Method of SDS-Page (Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis)**

Vilda Carlenia Wardani, Sri Pantja Madyawati, Poedji Hastutiek  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 205-211

**Comparison of Morula and Blastula Embryo Vitrification by Using Cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium**

Widjiati Widjiati, Epy Muhammad Luqman, Portia Sumarsono  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 212-223

**Effect of L-Arginine on the Thickness Iliac Arteries Wall Post Fogarty Balloon Embolectomy Catheter in Rabbit (*Oryctolagus cuniculus*)**

Gavrila Amadea Puspitarani, Ngakan Made Rai Widjaja, Hardany Primarizky  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 224-233

**Ellagic Acid Activity in Healing Process of Incision Wound on Male Albino Rats (*Rattus norvegicus*)**

Hardany Primarizky, Wiwik Misaco Yuniarti, Bambang Sektiari Lukiswanto  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 234-240

**Control and Preventive Study of Brucellosis by Using**

Rahmahani J, Handijatno D, Tyaningsih W, Suwarno Suwarno  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 241-251

**Hispathology of Coronary artery of male rat (*Ratus Norvegicus*) with high fat diet after being given ethanol extract of Indian acalypha (*Acalipha indica*, L)**

Kurnijasanti R, Winarti D, Wahyuni R.S., Puguh K, Setyabudi Setyabudi, Sukmanadi Sukmanadi, Sugihartuti R, Damayanti R, Hidayati N, Rahmawati K, Sudjarwo A.S.  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 252-265

**Immunohistochemical Detection of Porcine Reproductive and Respiratory Syndrome Virus Antigen in Formalin-Fixed, Paraffin-Embedded Tissues with Correlation to Clinicopathologic Data**

Lavina Gracia G. Manzano  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 266-277

**Anthelmintic Activity of *Ocimum sanctum* Linn. Leaves Ethanol Extract Against *Fasciola gigantica* in vitro**

Mesia Margi Mahardika, Sri Agus Sudjarwo, Setiawan Koesdarto  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 278-285

**Scabiosis in Rabbit**

Miyayu Soneta Sofyan, Doni Chrismanto  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 286-295



**Detection of Newcastle Disease Virus by Immunohistochemistry on the Brains of Laying Birds with Clinical Signs Torticollis and Curled Toe Paralysis**

Ocie Harum Wulan, Niken Yunita, Hastari Wuryastuty, Raden Wasito  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 296-307

**Implementation of Meniran Extract (*Phyllanthus Niruri* Linn) on the Performance of Broiler Chickens Infected by *Mycoplasma gallisepticum* Caused Chronic Respiratory Disease**

Sri Hidanah, Emy Koestanti Sabdoningrum, Retno Sri Wahjuni, Arimbi Arimbi  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 308-315

**Blood Parasite Infection Prevalence in Kampong Chicken Breeder's Group in Garut**

Djoko Legowo, Syifa Husnul Khotimah, Lucia Tri Suwanti  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 316-326

**Amino Acid Analysis of Fusion (F) Gene and Prediction of Epitope B-Cell Newcastle Disease Surabaya Isolate As Vaccine Candidate**

Indah Laili Rahmawati, Fedik Abdul Rantam, Wiwik Tyasningsih  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 327-337

**Mini Review : Liver Fibrosis Mechanism**

Wiwik Misaco Yuniarti, Hardany Primarizky  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 338-343

**The Nutrients Contents, Dry Matter Digestibility, Organic Matter Digestibility, Total Digestible Nutrient, and NH3 Rumens Production of Three Kinds of Cattle Feeding Models**

M. Anam Al-Arif, Lucia Tri Suwanti, AT Soelih Estoepangestie, Mirni Lamid  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 344-355

**Immunogenicity of Bone Graft Using Xenograft Freeze-Dried Cortical Bovine, Allograft Freeze-Dried Cortical New Zealand White Rabbit, Xenograft Hydroxyapatite Bovine, And Xenograft Demineralized Bone Matrix Bovine In Bone Defect Of Femoral Diaphysis White Rabbit Experimental Study In Vivo**

Ferdiansyah Ferdiansyah, Dwikora Novembri Utomo, Heri Suroto  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 356-368

**Genotyping Analysis of *Mycobacterium leprae* isolated in Water Environment of Leprosy Endemic Places in Lamongan, East Java**

Cita Rosita Sigit Prakoeswa, Nanny Herwanto, Ratna Wahyuni, Iswahyudi Iswahyudi, Dinar Adriaty, Indropo Agusni, Shinzo Izumi  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 369-377

**Iron Overload Reduces Cholesterol and Triglyceride Serum of Mice**

Devi Agustin Setiawati, Mas Rizky A.A. Syamsunarno, Pandji Irani Fianza, Nur Atik, Neni Anggraeini, Mohammad Ghozali, Ratu Safitri, Ramdan Panigoro  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 378-385

**Measurement of Alkaloids Achyranthes Aspera Linn Level Using Thin Layer Chromatography Method and High-Performance Liquid Chromatography**

Dewa Ketut Meles, Wurlina Wurlina, Dewa Putu Anom Adnyana  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 386-394

**Propolis Potential Toward the Amount of Lymphoblast and Spleen Diameter of Male Mice (Mus musculus)**

Werstant Adhityananda Rinaldhi, Eka Pramyrtha Hestianah, Sri Mumpuni Sosiawati, Lita Rakhma Yustinasari  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 395-403

**Identification the Gene Nucleotide Sequence of Outer Membrane Protein Aeromonas Hydrophilla Bacteria from East Java Local Isolates Using Polymerase Chain Reaction**

M. Gandul Atik Yuliani, Didik Handijatno, Sri Pantja Madyawati  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 404-412

**Postmortem Interval Estimation Time from Algormortis Temperature of Rats Expressed by MARS Model Approach**

Dwi M. Syabani, Hana Eliyani, Suharsono Suharsono, Fedik A. Rantam, Anwar Ma'ruf  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 413-421

**Aqueous Extract of Neem Leaves (Azadirachta Indica) Decrease Expression of Immunoglobulin E (IgE) and Interleukin 4 (IL-4) in Gingiva Tissue of BALB/c Mice Injected by Ovalbumine**

I Dewa Ayu Ratna Dewanti, I Dewa Ayu Susilawati, Pujiana Endah Lestari, Roedy Budirahardjo, Erawati Wulandari, Ristya Widi, Sunlip Wibisono  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 422-435

**Experimental Models Point Mutations In Plasmodium falciparum pfatpase6 Gene Exposed to Recuring Artemisinin In Vitro**

Lilik Maslachah, Yoes Prijatna Dachlan, Chairul A. Nidom, Loeki Enggar Fitr  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 436-442

**Sinensetin-Rich Fraction Solid Dispersion Inhibits Cancer Cell Cycle**

Lusiana Arifianti, Sukardiman Sukardiman, Mulja Hadi Santosa  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 443-449

**Blunted Expression of PPAR $\alpha$  in Mice with FABP-4 and -5 Deficiency under Acute Cold Exposure**

Mas Rizky A.A Syamsunarno, Mirasari Putri, Tatsuya Iso, Rini Widyastuti, Ramdan Panigoro, Masahiko Kurabayashi  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 450-459

**Increased Iron in Pediatric  $\beta$ -Thalassaemia Major Associates with CD3+, Not  $\gamma\delta$  Lymphocytes**

Mohammad Ghozali, Ulrike Panjaitan, Adi Imam Cahyadi, Reni Ghrahani, Lelani Reniarti, MRAA. Syamsunarno, Ramdan Panigoro  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 460-467

**Low Serum Cholesterol in Mice Pre-treated with Imperata cylindrica L. after Acute Olive Oil Gavage**



Neni Anggraeni, Mas Rizky A.A Syamsunarno, Ghina Rahmadianti Mukarromah, Almira Zada, Rima Destya Triatin, Yunisa Pamela, Diah Dhianawaty  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 468-475

**The Role Of IL-6 In TMPD-Treated Lupus Arthritis Mice**

Niken Indriyanti  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 476-485

**Immunopathological Approach for Avian Influenza Virus Detection in Brain of Laying Bird with Clinical Signs of Torticollis and Curled Toe Paralysis**

Niken Yunita, Ocie Harum Wulan, Hastari Wuryastuty, Raden Wasito  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 486-496

**The Efficacy of Permot (Passiflora Foetida Linn.) Leaves Crude Extract Ointment on the Healing of Skin of Rabbit with Scabies**

Poedji Hastutiek, Hana Eliyani  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 497-512

**The Effect of Sappan Wood Extract (Caesalpinia sappan), Wheat grass and Vitamin E Treatment on the Liver Structure of Iron overload of Rat (Rattus norvegicus)**

Ratu Safitri, Lelani Reniarti, Madihah Madihah, Lila Delia, Mas Rizky A.A Syamsunarno, Ramdan Panigoro  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 513-519

**The Effectiveness of Antibiotics and Hematopoietic Stem Cell Treatment in Periodontitis Rat Model Toward TNF  $\alpha$  Expression**

Retno Indrawati R, Indeswati Diyatri, Dwi Rahmawati  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 520-527

**Construction Hybrid immunoglobulin All Four Dengue serotype Using Mesenchymal Stem**

Rofiqul A'la, Rahaju Ernawati, Nunuk Dyah Retno L, Mufasirin Mufasirin, Anwar Ma'ruf, Fedik A. Rantam  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 528-535

**The Potential of Black Gluten and Red Rice in Rations on the Biological Values and Ideally Body Score of Healthy "Mini Rex Rabbit"**

Romziah Sidik  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 536-548

**Antiviral Activity Effect of Silver Nanoparticles (AgNps) Solution Against the Growth of Infectious Bursal Disease Virus on Embryonated Chicken Eggs with Elisa Test**

Rosa Pangestika, Rahaju Ernawati  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 549-556

**Identification and Characterization Indigenous of Lactobacillus sp from Bovine Rumen Fluid of Slaughterhouse**

Tri Nurhajati, Koesnoto Soepranionondo, Widya Paramita Lokapirnasari, Adriana Monica Sahidu  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 557-565



**Protective Effect of Propolis Extract Against Lead Acetate Toxicity in Mice (Mus Musculus) Testes**

Tuti Widawati, Sri Agus Sudjarwo, Herry Agoes Hermadi  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 566-578

**Detection of Antibiotic Residues and Concentration in Raw Milk from Lembang Small Holder Dairy Farm**

Virgianty Vivi  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 579-587

**Potency of Bacillus cereus WPL 415 to Increase Crude Protein and Decrease Crude Fiber of Animal Feed Stuff**

Widya Paramita Lokapirnasari, Adriana Monica Sahidu, Tri Nurhajati, Koesnoto Soepranianondo, Andreas Berny Yulianto  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 588-695

**Acute Toxicity Tests of Alkaloid Pare (Momordica Charanthia) Fruit on The Histopathology of Liver**

Wurlina Wurlina, Dewa Ketut Meles, Sunarni Zakaria, Imam Mustofa, Suhermi Susilowati, I Dewa Putu Anom Adnyana  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 696-602

**Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo**

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti, Erma Safitri  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 603-608

**Case Study : Dystocia on Beef Cattle in Kunir Regency of Lumajang District, East Java, Indonesia in 2015 and 2016**

Rosiana Febrianila, Widya P Lokapirnasari, Tjuk I Restiadi, Imam Mustofa, Herry A Hermadi, Erma Safitri  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 609-618

**The Effectiveness of Honey in Physiological Nacl to Maintain of Viability and Motility of Spermatozoa**

Elsa Agustina, Herry Agoes Hermadi, Hario Puntodewo S, Tatik Hernawati, Indah Norma Triana, Erma Safitri  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 619-626

**Utilization of Sumbawa Tropical Forest Honey Apis Dorsata to Improve Fertility of Indonesia Oriental Magpie Robin (Copsychus saularis) as Effort Animal Population Increase**

Abdullah Hasib, Risaldi Muhamad, Talita Yuanda Reksa, Alvina Ulimaz Artha, Erma Safitri  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 627-632

**Utilization of Honey Apis dorsata as Antiosteoporosis on Requirements of Bone Calcium Ash Density on Ovariectomized White Rat (Ratus norvegicus)**

Muhammad Huda Ramadhan Ibrahim, Abdullah Hasib Hasib, Siti Nur Rohmah, Salsabilla Abani, Samsi Yordan, Ira Sari Yudaniyanti  
[The Veterinary Medicine International Conference \(VMIC\)](#) | pages 633-641

**Increased Integrity of Plasma Membrane and Acrosome Cap Spermatozoa Limousin Cattle at Post Thawing in Frozen Media by adding Seawater Extract**

Nur Faidah, Tatik Hernawati, Mirni Lamid, Ismudiono Ismudiono, Tri Wahyu Suprayogi, Sri Mulyati

[The Veterinary Medicine International Conference \(VMIC\) | pages 642-649](#)

[\*\*The Relation of Body Temperature and Vaginal Cytology Examination in Time Artificial Insemination Rate Fat-tailed Sheep \(Ovis Aries\) in The District Sidoarjo East Java\*\*](#)

Rhendyka Prasetya Anggriawan, Suzanita Utama, Hana Eliyani  
[The Veterinary Medicine International Conference \(VMIC\) | pages 650-657](#)

[\*\*Effect of Laser Acupuncture Shoot on Ova Point of Male Mojosari Duck \(Anas platyrhynchos\) on The Number of Sertoli and Leydig Cells\*\*](#)

Yuanara AR Adikara, Abdul Samik, Ira S Yudaniyanti, Tatang S Adikara, Eka P Hestianah, Suzanita Utama  
[The Veterinary Medicine International Conference \(VMIC\) | pages 658-667](#)

[\*\*Insulin-Like Growth Factor-I \(IGF-I\) from Crossbred Pregnant MareSerum to Increase Follicle Number of Mice \(Mus musculus\)\*\*](#)

Abdullah Abdullah, Tjuk IRestiadi, Nunuk DR Lastuti, Tita Damayanti, Wurlina Wurlina, Erma Safitri  
[The Veterinary Medicine International Conference \(VMIC\) | pages 668-676](#)

[\*\*Morphological Identification Nematodes Tanqua tiara Found on Gastric Varanus salvator at East Java\*\*](#)

Alfiana Laili Dwi Agustin, Setiawan Koesdarto, Bambang Sektiari Lukiswanto, Lucia Tri Suwanti, Zainal Arifin, Emmanuel Djoko Putranto  
[The Veterinary Medicine International Conference \(VMIC\) | pages 677-683](#)

[\*\*Effect of Propolis on Spermatogenic Cells Number and Diameter of Seminiferous Tubules in Male Mice \(Mus musculus\)\*\*](#)

Dona Astari Nurkarimah, Eka Pramyrtha Hestianah, Retno Sri Wahjuni, Mas'ud Hariadi, Suryo Kuncorojakti, Herry Agoes Hermadi  
[The Veterinary Medicine International Conference \(VMIC\) | pages 684-693](#)

[\*\*The Effect of Blue Green Algae \(Spirulina platensis\) Extract in White Rat \(RattusNorvegicus\) Treated with Excessive Physical Exercise on Leydig Cell Number and Seminiferous Tubules Diameter\*\*](#)

Dimas Yuzrifar Rhavindra Lazuardi, Rimayanti Rimayanti, Hardany Primarizky, Sri Agus Sudjarwo, Suzanita Utama, Kadek Rachmawati  
[The Veterinary Medicine International Conference \(VMIC\) | pages 694-701](#)

[\*\*The Effect of Mahkota Dewa \(Phaleria macrocarpa\) Pulp Extract by Peroral Administration Toward The Percentage of Capacitation and Acrosome Reaction in Rat \(Rattus norvegicus\)\*\*](#)

Dhanang Estu Bagyo, Budi Utomo, Rudy Sukamto Setiabudi  
[The Veterinary Medicine International Conference \(VMIC\) | pages 702-711](#)

[\*\*Protection of Dayak Onion Tuber Extract \(Eleutherine Palmifolia\) Against Kidney Histopathological Appearance of Albino Male Rat Strain Wistar which was Induced by Alloxan\*\*](#)

Dwi Gayatri Nurcahyawati, Hani Plumeriastuti, Lilik Maslachah  
[The Veterinary Medicine International Conference \(VMIC\) | pages 712-717](#)

[\*\*Test Various Estrus Detection Device Against Pregnancy Rates on Dairy Cows in Cooperative Tunas Setia Baru Kabupaten Pasuruan\*\*](#)

Silvia Rani Andriyanti, Mas'ud Hariadi, Roesno Darsono, Pudji Srianoto



[The Veterinary Medicine International Conference \(VMIC\) | pages 718-726](#)

[Effect of Laserpuncture Shoot on Reproduction Point of Male Mojokari Duck \(Anas Platyrhynchos\) on The Numbers of Spermatogonium Cells and Seminiferous Tubules Diameter](#)

Melyandari Ayu Qomar, Rimayanti Rimayanti, Tri Nurhajati

[The Veterinary Medicine International Conference \(VMIC\) | pages 727-733](#)

[Cases of Reproduction Disorder in Beef Cattle of Modo District, Lamongan in 2015](#)

Azharuddin Anshoria, Tri Nurhajati, Budi Utomo

[The Veterinary Medicine International Conference \(VMIC\) | pages 734-741](#)

[The Application of Equine Chorionic Gonadotropin \(Ecg\) and Prostaglandin F2 \$\alpha\$  to Increase the Rate of Pregnancy in Bali Cattle at Buleleng, Bali](#)

Yugenthri A/P Chandran, Herry Agoes Hermadi, Eka Pramyrtha Hestianah

[The Veterinary Medicine International Conference \(VMIC\) | pages 742-752](#)

[Morfometry Study of Hemipenis Biawak Air Varanus Salvator on Length Measurement of Snouth Vent Length \(Svl\) Andbody Weight](#)

Ilham Adi Kusuma, Dicky Beo Alfiyanto, Pudji Sianto, Nurdianto Triakoso, Djoko Legowo

[The Veterinary Medicine International Conference \(VMIC\) | pages 753-762](#)

[Effect of Polygonum Minus \(Knotweed\) Leaves Extract on the Histopathological Changes of Kidney in Mice \(Mus Musculus\) Induced by Mercuric Chloride](#)

Winni Aprianti, Thomas Valentinus Widiyatno, Sri Agus Sudjarwo

[The Veterinary Medicine International Conference \(VMIC\) | pages 763-775](#)

[Skin Stem Cell Resource Potential for Peripheral Nerve Repair Due to trauma of post regional anesthesia](#)

Sumartono Christrijogo, Fedik A Rantam, Eddy Rahardjo, Martia R Tacharina

[The Veterinary Medicine International Conference \(VMIC\) | pages 776-781](#)

[Public Awareness in ensuring Animal Originated Food Safety : A Review on "One Health" Approach in Veterinary Medicine](#)

A. T. Soelih Estoepongastie

Corresponding Author: Widya  
Paramita Lokapirnasari  
widyaparamitalokapirnasari@  
gmail.com

Received: 03 October 2017

Accepted: 10 October 2017

Published: 29 November 2017

**Publishing services provided  
by Knowledge E**

© Widya Paramita Lokapirnasari  
et al. This article is distributed  
under the terms of the [Creative  
Commons Attribution License](#),  
which permits unrestricted use

# Protein and Decrease Crude Fiber of Animal Feed Stuff

Widya Paramita Lokapirnasari<sup>1</sup>, Adriana Monica Sahidu<sup>2</sup>, Tri Nurhajati<sup>1</sup>,  
Koesnoto Soepranianondo<sup>1</sup>, and Andreas Berny Yulianto<sup>3</sup>

<sup>1</sup>Department of Animal Husbandry, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

<sup>2</sup>Department of Marine, Faculty of Fisheries and Marine, Airlangga University, Surabaya, Indonesia

<sup>3</sup>Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Surabaya, Indonesia

## Abstract

This research aims to identify isolate as a probiotic candidate derived from liquor rumen of local beef cattle and to know the ability of isolates as biofermentor on basal feed to the changes in the nutrient value. The selected samples were obtained from a slaughterhouse in Surabaya. This study consisted of two stages. The first stage was the identification of bacteria through the

VMIC2017  
The Veterinary Medicine International Conference 2017  
Volume 2017



and redistribution provided that  
the original author and source  
are credited.

Selection and Peer-review under  
the responsibility of the VMIC  
Conference Committee.

## Conference Paper

# Potency of *Bacillus cereus* WPL 415 to Increase Crude

test of morphology, Gram staining, biochemical, resistance to acidity and 16S rDNA sequencing. The second stage was a test of the ability of the isolates on the nutrient of basal feeds by fermentation for three days in an aerobic condition. Based on the findings of the first phase, it has been identified that probiotic bacterium rods, motility positive, Gram-negative, have viability at pH 2 and pH 3 for 90 minutes and 24 hours and have the ability to ferment lactose, sucrose, galactose, ribose, cellobiose and xylose. Furthermore, based on test results of 16S rDNA sequencing, the probiotic bacterium was identified as *Bacillus cereus* WPL 415. Based on the research results at the second stage, *Bacillus cereus* WPL 415 at doses of 0.25% and 0.5% could improve the nutrient content of the basal feed. The results of the proximate analysis revealed that there was an increase in crude protein content of 6.78% until 8.12% compared to the control and was able to lower the crude fiber content of 15.19% and 17.40% compared to the control. Based on these results it can be concluded that *Bacillus cereus* WPL 415 from local beef cattle can be used as a probiotic candidates to improve the quality of animal feed.

**Keywords:** *Bacillus cereus*, probiotic, crude protein, crude fiber.

## OPEN ACCESS

**How to cite this article:** Widya Paramita Lokapirnasari, Adriana Monica Sahidu, Tri Nurhajati, Koesnoto Soepranianondo, and Andreas Berny Yulianto, (2017), "Potency of *Bacillus cereus* WPL 415 to Increase Crude Protein and Decrease Crude Fiber of Animal Feed Stuff" in *The Veterinary Medicine International Conference 2017*, KnE Life Sciences, pages 579–587. DOI 10.18502 /kls.v 3i6.1185



## 1. Note

Please read these instructions carefully and print them. At the end of the instructions you will find a button that removes this text and prepares the document for your text. (Note that this button may not work properly if you change in any way this text.) Use the styles, fonts and point sizes as defined in this template, **but do not change or redefine** them in any way as this will lead to unpredictable results.

## 2. Introduction

Probiotics are beneficial living microorganisms, either mono culture or mixed cultures that if applied to humans and animals will provide beneficial effects for the host by improving the properties of the indigenous flora, improving the health status of man or animals [1] and have the ability to modulate the balances and activities of the gastrointestinal (GI) microbiota [2]. Probiotic should be able to stimulate growth, improve feed conversion ratio and inhibit enteropathogens, without causing any undesirable effect. In the application process, probiotics must survive the stress produced during manufacturing, storage and administration at farm conditions [3]. Several strains have been used as probiotics i.e. *Lactobacillus*, *Pediococcus*, *Bacteroides*, *Bifidobacterium*, *Bacillus*, *Streptococcus* and *Escherichia coli*, alone or consortiated [4].

Based on the results of characterization, it was identified that *Bacillus* species ( *Bacillus cereus*, *Bacillus clausii*, *Bacillus pumilus*) had potential as a probiotic based on the ability of colonization, immunostimulant, and antimicrobial activity [5]. *Lactobacillus plantarum* and *Bacillus spp.* spores have been reported to decrease the amount of Vibrionaceae in rotifers fed with these additives, and subsequently increase weight and survival of turbot larvae [6]. Although some strains of *Bacillus* species have been used as probiotics, but the information related to the advantages of using *Bacillus* have not been widely reported, thus, this study aims to carry out the exploring bacteria as a probiotic candidate sourced from isolated indigenous of local beef cattle from slaughterhouses in Surabaya.

## 3. Materials and Methods

The materials used are fresh gut of local cattle from abattoirs, alcohol 70%, medium selective MRSB (de Man Rogosa Sharpe Broth), MRSA (de Man Rogosa Sharpe Agar) (OXOID),

medium Luria Bertani (LB) (MERCK), Gram stain (Crystal Violet, Lugol, alcoholacetone, and safranin), physiological NaCl, 0.1 N HCl. **Sample Preparation.** The part of intestinal organs were rinsed with sterile distilled water and crushed using a mortar and weighed as much as 1 g and put into a sterile physiological NaCl solution and diluted with multilevel dilution ( $10^{-1}$  -  $10^{-6}$ ). A total of 1 ml of  $10^{-6}$  dilution was inoculated on MRSA (Man Ragosa Sharpe Agar) medium, then incubated for 24-48 hours at 37° C. **Purification of Bacteria Probiotic Candidate.** Purification was done by selecting a colony on the surface and then inoculated on the surface of MRSA ( Man Ragosa Sharpe Agar) medium with scratch method to obtain a separate colony. It was incubated at 37° C for 2x24 hours. The purification step could be done 2-3 times to obtain pure isolates. Furthermore, the isolate was inoculated on MRSA medium slant as stocks for further testing. The observation of cell morphology was done by using the Gram stain. **Test of Resistance on Acidity (pH).** The resistance test on acidity was done using MRS broth medium supplemented with 0.1 N HCl to obtain pH 23 (according to the pH of the stomach). As much as 1 ose each bacterial isolates was taken from the stock culture and was inoculated on MRSB-HCl medium. After that, it was incubated for 2x24 hours at 37° C. If there was a growth of bacteria on MRSB-HCL medium, it showed positive results, and it showed negative results if there was no growth of bacteria on the MRSB-HCL medium. **Identification of the gene encoding 16S rDNA.** DNA isolation was using the method of Ausubel [7]. **The second stage.** In the second phase of the study was carried out a test of *B.cereus* WPL 415 ability against basal feed through the fermentation process. The study was divided into three treatments with each of eight repetitions. The treatment consisted of: P0: P0: feed without *B.cereus* WPL 415, P1: feed + 0.25% *B.cereus* WPL 415, and P2: feed + 0.50% *B.cereus* WPL 415. The fermentation of basal feed was done by adding a solution of inoculant appropriate treatment dose, then dissolved in a 3% drop and 20% non-chlorine water. After the solution was mixed homogeneously, sprayed on basal feed. Fermentation was done under anaerobic conditions using a plastic bag as a silo for 3 days. After the fermentation period ended, the plastic was opened, then proximate analysis was performed to determine changes in the nutritional content of crude protein and crude fibre. Data were analysed by analysis of variance. If the results were significantly different ( $P < 0.05$ ) between treatments, the analysis was proceeded by Duncan's multiple range test [8].

## 4. Results and Discussion

Based on identification results, the isolate obtained from the small intestine of cattle, had the following characteristics: a rod-shaped cells, Gram negative and positive



T 1: Biochemistry Test Results of WPL 415.

Biochemistry Test	reaction	Biochemistry Test	reaction
Lactose	+	Galactose	+
Sucrose	+	Cellubiose	+
Gluconae	+	Rafinose	+
Ribose	+	Manitol	+
Xylose	+	Ramnose	-
Arginine	-	Esculine	+
Arabinose	-		

motility. The results of biochemical tests isolates obtained in this study demonstrated the ability of fermentation as listed in Table 1.

The arrangement of obtained nucleotide isolates, further was identified with the program BLAST (Basic Local Alignment Search Tool) in [www.ncbi.com](http://www.ncbi.com) and isolates obtained which had some similarities with the arrangement of nucleotide similarity level 92% -88%. (*Bacillus cereus* ATCC 14579, identity 92%, sequence ID ref|NC\_004722.1; *Bacillus cereus* Rock4-18, identity 92%, sequence ID ref|NZ\_CM000735.1; *Bacillus cereus* AH621, identity 92%, sequence ID ref|NZ\_CM000719.1; *Bacillus megaterium* DSM319, identity 89%, sequence ID ref|NC\_014103.1; *Bacillus licheniformis* ATCC 14580, identity 88%, sequence ID ref|NC\_006270.3; *Bacillus hemicellulosilyticus* JCM 9152, identity 88%, sequence ID ref|NZ\_BAUU01000088.1; *Bacillus amyloliquefaciens* DSM7, identity 88%, sequence ID ref|NC\_014551.1; *Bacillus subtilis* subsp. *subtilis* str. 168, identity 88%, sequence ID ref|NC\_000964.3; *Bacillus subtilis* subsp. *spizizenii* TU-B-10, identity 88%, sequence ID ref|NC\_016047.1; *Bacillus cellulosilyticus* DSM 2522, identity 88%, sequence ID ref|NC\_014829.1)

This research showed new isolate namely *Bacillus cereus* WPL 415. Based on the research results of Navinchandran, had been identified through sequencing of 16 S rRNA, a probiotic bacterium *Bacillus cereus* from the gut of wild shrimp *Penaeus monodon* [9]. The probiotic bacterium had antagonistic activity against pathogenic bacteria in shrimp as well as having the ability to produce extracellular enzymes. Probiotic *B. cereus* at a concentration of 0.4% / 100 g of feed was efficient in stimulating the growth (specific growth rate / SGR of  $4.40 \pm 0.179\%$

and a better feed conversion ratio / FCR of  $1.27 \pm 0081$ ) and immunity (total count haemocyte, lysozyme activity, plasma protein concentration and bactericidal activity) in shrimp.

T 2: Viability of the bacteria *Bacillus cereus* WPL 415 at pH 3 and pH 4.

Viability of the bacteria <i>Bacillus cereus</i> WPL 415								
Time	MRS	Agar	(control)	MRS	Agar	pH 3	MRS	Agar
	CFU/ml			CFU/ml			CFU/ml	pH 4
90 minutes								
			$1.4 \times 10^8$			$1.0 \times 10^8$		$2.1 \times 10^8$
			$2.1 \times 10^8$			$1.2 \times 10^8$		$2.0 \times 10^8$
24 hours			$7.5 \times 10^8$			$1.0 \times 10^8$		$3.6 \times 10^8$
			$6.6 \times 10^8$			$0.4 \times 10^8$		$4.8 \times 10^8$

Among the various species of probiotics, those belong to the genus of *Bacillus* which had the advantage that, due to their capacity to sporulate, they survive at ambient temperatures as well as during desiccation by methods that involve; moderate heating, such as spray dryers, avoiding the use of lyophilization or other expensive technologies [10]. *B. cereus* strains were shown to persist in the mouse gastrointestinal tract for up to 18 days post administration, demonstrating that these organisms had some abilities to colonize. The spores of one *B. cereus* strains were extremely sensitive to simulated gastric conditions and simulated intestinal fluids [11].

*Bacillus* is able to grow on various kinds of sugar, these isolates also has a cellulolytic activity shown on its ability to degrade trinitrophenyl-carboxymethyl cellulose and growth on medium containing glucose cellobiose or produce the largest cellulolytic activity. Cellulolytic activity is not generated until the stationary phase of growth. Maximum cellulolytic activity test occurs at pH 4.8 and a temperature of 58 °C [12].

The research results in Table 2 indicated that the isolates of *Bacillus cereus* was able to sustain life in the acidic conditions of pH 3-pH 4. It was shown from the comparison with the controls, which in 90 minutes showed the viability of control about  $1.4 \times 10^8$  -  $2.1 \times 10^8$  CFU / ml. On the condition of acid pH 3 for 90 minutes, *Bacillus cereus* isolates demonstrated the viability of  $1.0 \times 10^8$  -  $1.2 \times 10^8$  CFU / ml, whereas at pH 4 for 90 minutes showed the viability of  $2.0 \times 10^8$  -  $2.1 \times 10^8$  CFU / ml. Later in the control condition for 24 hours, *Bacillus cereus* isolates demonstrated the viability of  $7.5 \times 10^8$  -  $6.6 \times 10^8$  CFU / ml, whereas at pH 3 for 24 hours



demonstrated the viability of  $0.4 \times 10^8$ – $1.0 \times 10^8$  CFU / ml. On the condition of acid pH 4 demonstrated the viability of  $3.6 \times 10^8$ – $4.8 \times 10^8$  CFU / ml.

Resistance to acidity test results showed that isolates of *Bacillus cereus* was considered able to survive through the digestive tract system that had a low pH conditions, so as to reach the intestine to be able to do activities to maintain the balance of microflora. *Bacillus cereus* has the ability to survive in the intestinal tract and during manufacturing, such as interaction with enteropathogens, resistance to heat and to variation of pH.

T 3: *B. cereus* fermentation influence on the levels of crude protein and crude fibre of feed.

Treatment	Crude Protein $\pm$ SD	Crude Fiber $\pm$ SD
P0 (0% <i>B.cereus</i> WPL415)	17.99 <sup>□</sup> $\pm$ 0.07	6.78 <sup>□</sup> $\pm$ 0.68
P1 (0.25% <i>B.cereus</i> WPL415)	19.21 <sup>□</sup> $\pm$ 0.02	5.60 <sup>□</sup> $\pm$ 0.21
P2 (0.50% <i>B.cereus</i> WPL415)	19.45 <sup>□</sup> $\pm$ 0.5	5.75 <sup>□</sup> $\pm$ 0.25

□ different superscripts in the same column, shows that there were significant differences (p < 0.05).

Variations in pH of the gastrointestinal tract could affect the viability of the isolate as a probiotic candidate. *Bacillus* genus have strong adaptability to diverse conditions and that several species produce highly resistant spores; they have been isolated from fish [13;14]. Based on the results of statistical analysis, the use of *B. cereus* WPL 415 in the fermentation basal feed showed significant differences among treatments on the content of crude protein and crude fibre (Table 3).

The results of the proximate analysis the crude protein content was the lowest for the P0 treatment (control, without the use of *B. cereus* WPL 415), while the use of 0.25 % *B. cereus* WPL 415 and 0.5% crude protein were able to increase the content of 6.78 % until 8.12% compared with control. The highest results of the proximate analysis of crude fibre content was at P0 treatment (control, without the use of *B. cereus* WPL 415) , while the use 0.25% *B. cereus* WPL 415 and 0.5% were able to lower crude fibre content of 15.19% to 17.40% compared to control patients. The results of this study indicate that WPL 415 *B. cereus* was able to increase the nutrient of the forage. The increase in crude protein content was due to the increased biomass of single cell protein *B. cereus* WPL 415. In the fermentation process, nutrients were available for the media used for the breeding biomass of *B. cereus* WPL 415, thus, it increased the number of microbe biomass, in which the increase was detected from the results of proximate analysis of crude protein.

The use of *B. cereus* WPL 415 in the fermentation basal feed could also reduce the content of crude fibre. It was supported by the test results that demonstrates the ability of isolates to ferment crude fibre, namely xylose and cellobiose. Cellulose fraction was the biggest component of a constituent of plant cell walls that were very difficult and even could not be digested by monogastric digestive enzymes, so that the cellulose must be broken down first into low molecular weight compounds such as mono, di and tri saccharides. The degradation involved a complex of cellulase enzymes produced by microbes are endo-beta-glucanase and beta-glucosidase.

Cellulase enzyme is a complex enzyme consists of a group of enzymes that work synergistically to degrade cellulose i.e. 1, 4- $\beta$ -D-glucan-4-glucanohydrolases or endoglucanase; 1, 4- $\beta$ -D-glucan glucanohydrolases or exoglucanases and  $\beta$ -glucoside glucohydrolases or cellobiase. Endoglucanase enzyme randomly cut the internal amorphous on the chain of 1, 4- $\beta$  polisaccharides cellulose into cellulo-oligosaccharides. Exoglucanases enzyme take a role in glucose unit at the polar end of the reduction or nonreduction of the chain of cellulo-oligosaccharides produces Cellobiose ( disaccharide ). The  $\beta$ -Glucosidases enzyme hydrolyse Cellobiose into glucose [15]. Supplementation of 0.20% -0.40% rumen bacterial culture cellulolytic isolates buffalo can improve the body weight gain and feed efficiency of ducks. This is due to the addition of a bacteria culture acts as probiotics can stimulate the synthetic enzyme digestion enhancing the utilization of nutrients [16].

Based on the results of the study [17], the use of cow's rumen fluid was very potential as inoculant that contained of high nutrients and ready fermentable microbial and fibre degrading enzymes. The use of rumen fluid was capable of producing an inoculant with high nutrient and microbiology that were effective to be used as a starter. The high population of microbial inoculant and the support by the substrate degradation ability was high, and the high activity of cellulose and xylanase enzymes had been able to decrease crude fibre content of the ration so that the nutrient of the ration fermented inoculants' quality produced increases. The improvement quality of the nutrient of the fermented feed gave a positive response to the increase in the digestibility [18]. The results of other studies indicate the existence of extracellular products produced by *Bacillus* sp which indicates strongly inhibited the growth of *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from diseased fish. APIZYM

Enzyme assays showed that both bacteria have esterase lipase, leucine arylamidase, acid phosphatase, lipase and Naphthol-AS-BI-phosphohydrolase activities [19].

## 5. Conclusion

Based on these results it can be concluded that *Bacillus cereus* WPL 415 from local beef intestine can be used as a probiotic candidates to improve the nutrient value of animal feed stuff.

## Acknowledgments

The author would like to thank to the Rector of the University of Airlangga, Chairman of the Institute for Research and Inovative of Airlangga University, and Ministry of Research, Technology and Higher Education who has funded this research on Commodity Research Universities (PUPT). Researchers also would like to thank to all who has helped this research.

## References

- [1] R.Havenaar, B. Ten Brink, and J. H. Huis, Selection of strains for probiotic use. In Probiotics. (1992) 209-224. Springer Netherlands.
- [2] Y. Uyeno, S. Shigemori and T. Shimosato. J. Microbes and environments, 30(2)(2015)126-132.
- [3] C.Gil-Turnes, A.F.D.Santos, F.W.D. Cruz and A.V.Monteiro. CenBiot. Revista de microbiologia, 30(1) (1999)11-14.
- [4] R.Fuller, Probiotics. J. Appl. Bacteriol. SS, 1S-7S, 1986.
- [5] L.H.Duc, H.A.Hong, T.M.Barbosa, A.O.Henriques, and S.M.Cutting. Applied and environmental microbiology, 70(4)(2004)2161-2171.
- [6] J. Günther, and M, R.Jiménez. Revista de biología tropical, 52(4)(2004)937-943.
- [7] F.M.Ausubel, R. Brent, R.E.Kingston, D.D.Moore, J.G Seidman, J.A.Smith and K.Struhl, 2003. Current Protocols in Molecular Biology. 2<sup>nd</sup> ed. John Willey & Sons, New York.
- [8] G. D.Steel and J. H. Torrie.. Principles and procedure of statistics. New York: McGraw Hill Book Company.(1990).
- [9] M.NavinChandran,P.Iyapparaj,S.Moovendhan,R.Ramasubburayan, S.Prakash, G.Immanuel and A.Palavesam. Fish & shellfish immunology, 36(1)(2014)38-45



- [10] R.Havenaar, B. Ten Brink and J.H.Huis, 1992. Selection of strains for probiotic use. In Probiotics (pp. 209-224). Springer Netherlands.
- [11] L.H.Duc, H.A.Hong, T.M.Barbosa, A.O.Henriques and S.M.Cutting. Applied and environmental microbiology, 70(4)(2004)2161-2171.
- [12] L.M. Robson and G.H.Chambliss. Applied and Environmental Microbiology, 47(5) (1984)039-1046.
- [13] S.M.Aly, M.F.Mohamed and G.John, Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). Aquaculture research, 39(6):647-656(2008).
- [14] I. Murillo and L.Villamil, Journal of Aquaculture Research & Development. 2011.
- [15] L.R.Lynd, P.J. Weimer, W.H van Zyl and I.S.Pretorius. Microbiol. Mol. Biol. Rev.66(3) (2002)506-577.
- [16] N.W.Siti, I.G.N.G. Bidura and I.A.P.Utami. Journal of Biological and Chemical Research, 33(2016)214-225.
- [17] D.N. Kamra, Rumen microbial ecosystem. Current science, 89(1):24-135(2005).
- [18] I K.P. Nugraha, I.K.Sumadi, I.M. Mudita and I.W.Wirawan. e-jurnal Peternakan Tropika, 3(2)(2015)44-258.
- [19] I.Murillo and L.Villamil,. J. of Aquaculture Research & Development. 2011(2011).